REMARKS

Claims 1-21, 43-46 and 48-65 are pending. Claims 8, 13 and 44-46 have been amended in response to the Examiner's objections to clarify the subject matter claimed. Claim 65 has been added drawn to additional particular cell types and finds support, for example, in paragraph 66 of the specification. Applicant reserves the right to prosecute any withdrawn or cancelled subject matter in one or more continuation or divisional applications.

Applicants thank the Examiner for withdrawal of the claim objections, objections to the specification and rejection under 35 USC §101.

REJECTION UNDER 35 U.S.C. §102(A)

The Examiner has rejected claims 1-16, 51-59, 61 and 63-64 under 35 USC §102(a) as anticipated by Lai, et al. (February 2002) *Science* 295:1089-1092. Apparently, the Examiner has based this rejection on the theory that the pigs produced by Lai inherently anticipated the presently claimed pigs and their tissues and cells. The Examiner has shifted the burden of proof to the Applicant to show that the pigs were not homozygous.

The claims require that the pigs "lack any expression of functional α-1,3-galactosyltransferase." The Applicant need not go beyond the confines of the Lai reference to show that the pigs produced by Lai were heterozygous α-1,3-GT gene knock-outs and were *not* lacking α-1,3-galactosyltransferase (α-Gal) protein expression. Specifically, Lai et al. stated that any abnormalities seen in the piglets are unlikely to result from the genetic manipulation because "there is not a consistent phenotype and only one allele has been targeted" (see page 1091). Furthermore, Lai et al. noted that "[t]he **next step** will be to create α-1,3-galactosyltransferase-null" pigs (see page 1092, emphasis added). The fact that the Lai piglets still expressed functional α-Gal is supported by later studies coming out of the same group. For example, in 2004, Kolber-Simonds, et al. reported the production of α-Gal negative swine through further manipulation of one of the heterozygous piglets produced in Lai, et al. (see Kolber-Simonds, et al. (2004) *PNAS* 101:7335-7340, attached). In the later article, the group provided a direct comparison of the expression of Galα-1,3-Gal epitopes on cells from heterozygous animals

obtained in the Lai studies and the homozygous animals later produced, showing that the earlier developed heterozygous animals still produced functional α-Gal (see Figures 6 and 7).

This is not a case in which the chemical composition of the reference is identical to that claimed. The claimed animals are pigs that lack expression of functional α-Gal. The animals described in Lai were merely heterozygous for *one allele* of the α-Gal gene, and had no further manipulation. These pigs therefore still expressed the protein. There is no suggestion in the reference that *any* further processing of the cells took place to eliminate or modify expression of the other allele, the reference specifically identifies production of α-Gal null pigs as a *future goal*, and the animals that were produced in the reference, when tested, were *proven* to fall outside the scope of the claims. The Examiner is respectfully requested to withdraw this rejection.

REJECTION UNDER 35 U.S.C. §102(B)

The Examiner has maintained the rejection of claims 8, 13 and 48-50 under 35 U.S.C. §102(b) over Gustafsson et al. (U.S. Patent No. 6,153,428). The Examiner asserts that Gustafsson teaches a tissue "of a pig" that lacks expression of functional α-Gal. The Examiner also asserts that she is interpreting "cells" to read on "tissues." Applicant disagree that Gustafsson anticipates the claimed invention because Gustafsson does not teach any tissues or cells *obtained from a pig* that lacks expression of functional α-Gal, as recited in the pending claims. The Examiner appears to agree with this as she has limited her rejection to cell and tissue claims.

First, Applicants note that Gustafsson did not actually make *any* cells lacking expression of α-Gal. In fact, Gustafsson provides only very broad prophetic examples for possible methods of making such cells. To the Applicants knowledge, no one has ever made porcine cells lacking expression of functional α-Gal using the techniques outlined in Gustafsson.

Furthermore, as noted previously, the present invention is based on the first successful birth of viable pigs that lack any expression of functional α-Gal. The Examiner has rejected the previous arguments apparently based on the idea that α-Gal null cells are the same, whether they have been derived from an α-Gal null pig or simply manipulated in culture. This is not the case.

Cells derived from an animal that lacks functional α-Gal are not the same as cells in culture in which the gene has been inactivated. As noted in claims 48-50, the cells are to be used as a supplement or replacement for recipient cell, tissues or organs. Homozygous knock-out cells made in culture cannot be used in this manner. To provide sufficient cells to serve as replacements or supplements, these cells would have to be grown for extended periods of time in culture, leading to detrimental mutations, karyotypic abnormalities, possible contamination and a potential for carcinogenesis. Such cells are not useful as therapeutic agents.

Applicants note that previously pending claims 8 and 13 were not expressly limited to cells *obtained from* the α-Gal negative pigs. To assist the Examiner, claims 8 and 13 have been amended to provide that the cells are "obtained from a non-naturally occurring pig." This is an important limitation that is *not* met by Gustafsson.

Applicants believe that these amendments and arguments overcome the Examiner's rejections. However, should the Examiner believe that a different amendment or further language to clarify this point would facilitate allowance, she is asked to contact the Applicants representative to discuss these.

REJECTION UNDER 35 U.S.C. §102(E)

The Examiner also maintained the rejection of claims 1-16, 43-44, 46 and 48-49 under 35 U.S.C. §102(e) as anticipated by Denning et al. (U.S. Patent No. 7,126,039).

Applicants again note that Denning, much like Gustafsson, merely discusses the desired production of α-Gal null pigs and fails to teach the animals, cells and tissue of the present invention. The only examples that are described as having been reduced to practice are for sheep *cells*.[1] There is no example in Denning showing that *any* viable transgenic animal was actually produced.

The Examiner has maintained the rejection on the theory that the claims are not limited to the working examples, in which one allele of the α-Gal gene was knocked out and the other

[1] Example 4 describes the production of a sheep cells heterozygous for the alpha-1,3-GT gene

contained a particular point mutation. Therefore, the Examiner contends that the present claims are not enabled for their full scope and thus the prior art references can be cited.

Applicants are confused as to the Examiner's reasoning on this matter, but assume that the Examiner is basing this rejection on the a theory that the reference enables α-Gal negative animals to the same extent as the present teachings. As previously noted, no one was able to produce pigs lacking α-Gal expression before the present invention in part because a person of ordinary skill would have lacked any expectation of success (see for e.g. (see e.g. Ayares et al. (2001) *Graft* 4:80-85; Sharma et al. (2003) *Transplantation* 75:430-436; Porter & Dallman (1997) *Transplantation* 64:1227-1235; Galili, U. (2001) *Biochimie* 83:557-563**).** Contrary to the Examiner's assertion that the present application is not enabled, the Applicants point to at least pages 18-45 of the specification which provide a number of methods that, if followed, result in a pig that lacks expression of functional α-Gal with only routine experimentation. Applicants provided pigs made by one described method, and α-Gal negative pigs were also later provided by Kolber-Simonds, et al. following a different method that was substantially outlined on page 34 of the present specification. The present specification therefore provides *at least two* methods that have been proven to produce viable pigs lacking any expression of functional α-Gal.

As previously noted, prior to this invention, it was generally believed that the disruption of both alleles of the α-Gal gene would be lethal or extremely detrimental to the pigs (see e.g. Ayares et al. (2001) *Graft* 4:80-85; Sharma et al. (2003) *Transplantation* 75:430-436; Porter & Dallman (1997) *Transplantation* 64:1227-1235; Galili, U. (2001) *Biochimie* 83:557-563). Indeed, many experts in the field expressed serious doubts as to whether homozygous α-Gal knockout pigs would be viable at all, much less develop normally. *Such concerns were expressed up until the animals of the present invention were produced.* All the prior art provided merely prophetic examples of transgenic pig production that one of ordinary skill in the art would not have considered pursuing as part of ordinary experimentation in the context of the deep reservations that were held against the ability to produce these animals. Without the teaching provided by the present inventors, no one would have expected these animals to survive and be useful to harvest tissues, organs or cells. However, once the present inventors proved that pigs lacking α-Gal were viable and could be used for production of xenotransplants, these

reservations vanished. No pig that lacks functional α-Gal expression existed prior to the present invention. Applicants respectfully request withdrawal of this rejection.

The Examiner has also rejected claims 1-8, 13, 17-18, 43, 48, 60 and 62 under 35 U.S.C. §102(e) over Hawley, et al. (US 2006/0242722). Applicants point the Examiner to the priority document of Hawley, US Provisional Application No. 60/403,405, which does not include the production of viable animals using *any* technique, and thus does not provide any indication that this reference anticipates the claimed invention more than the other references cited. Furthermore, Applicants note that the pigs in Hawley post-date the pigs produced by the present inventors. As noted in the attached New Scientist news release dated January 13, 2003 and entitled "Mini-pig clone raises transplant hope", the pigs from Hawley's lab at Immerge were born in November, 2002 whereas the pigs that are the subject of the present invention were born in July, 2002. The article notes that "[h]owever, Goldie is not the first double-knock-out pig to be cloned." Hawley therefore does not predate the present application for the relevant information, which is the production of viable pigs lacking any expression of functional α-1,3-galactosyltransferase.

Applicants respectfully request that the Examiner withdraw the above rejections as no prior art reference provides pigs that lack α-Gal expression by any method, but merely provide prophetic examples of production of such animals. As discussed previously and again below, the production of any pigs lacking α-Gal would have been considered highly speculative prior to the present invention because one of skill in the art would have believed that the pigs would be developmentally compromised and that the lack of *a major sugar,* specifically Gal α-1,3-Gal on the surface of all pig cells would have been lethal.

REJECTION UNDER 35 U.S.C. §103

The Examiner has rejected claim 1-18, 51-59, 61 and 63-64 under 35 U.S.C §103(a) over Lai in view of Straham, et al. (1996) *Frontiers in Biosc.* 1:e34-41. The Examiner asserts that, although Lai produced only heterozygous pigs, Straham provides the motivation to breed

homozygous knock out pigs because it indicates that the Gal-α-1,3-Gal epitope was likely a major target for human anti-pig antibodies.

As noted previously, Applicants do not assert that they were the first to believe that α-Gal null animals would be useful *if it were possible to make viable animals*, merely that they were the first to overcome the expectation that these animals were non-viable and thus were the first to pursue techniques that would lead to successful production of living animals that lacked functional α-Gal. Without a reasonable expectation that a combination of references would lead to success, the ordinarily skilled artisan would not combine these references. Applicants respectfully request withdrawal of this rejection.

REJECTION UNDER 35 U.S.C. §112

The Examiner has rejected claims 19, 44-46, 49-50 as lacking enablement for the full scope of the claims. Specifically, the Examiner has rejected these claims as not enabled for pigs made using embryonic stem cell technology or produced using conventional nuclear transfer technology to make DKO cells.

Solely in the interest of prosecution, claims 44-46 have been amended to identify that the cells are fibroblast cells. As previously acknowledged, embryonic stem cell (ES) knock out technology is not useful in pigs and these claims were not intended to be directed at ES technology. Applicants believe this amendment overcomes the issues of the ES technology and addresses much of the Examiner's remaining issues detailed on pages 13-15 of the office action, insofar as the Examiner asserts that the method claimed using Toxin A is exemplified only for fibroblast cells, not for an embryonic stem cell. Furthermore, the Examiner asserts that homozygous genetic knock-out technology was not enabled by the specification. Although Applicants do not agree with the Examiner, claims 44-46 and 49-50 have been amended to recite that the cells lack expression of α-Gal, not that the cells have a homozygous knock out of the gene.

Applicants believe these amendments address the Examiner's concerns. Should the Examiner require additional amendments, she is asked to contact the undersigned representative to discuss these.

Applicants believe no additional fees are required with this response. Should the Examiner determine otherwise, the Commissioner is authorized to charge any underpayment of fees to Deposit Account No. 11-0980.

Respectfully submitted,

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